# DIRECTIONS FOR CULTURING PLASMA

## I. ROUTINE TO BE FOLLOWED IN EACH OF THE LABORATORIES OF THE COOPERATING HOSPITALS.

When plasma has been removed from 8 - 12 recipient bottles, (depending upon the size of the pooling flask), 10cc. of each pool is withdrawn into a sterile pipette for culture.

### A. Aerobic cultures:

5 cc. of the plasma from each pool is inoculated into an 8x1 inch tube containing 35 cc. of a modified Holman's 0.2% dextrose cooked meat medium. This medium is prepared as follows:

Stir 1 lb. of very fresh lean chopped beef heart into 1 litre of water (distilled preferred). This infusion should be left in refrigerator overnight.

Boil vigorously for 15 minutes. Strain through cheese cloth and restore to original volume with distilled water.

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| Add Neopeptone (Difco) | 1% | (10 grams) |
| NaCl | 0.5% | ( 5 grams) |

and stir until dissolved.

For Dextrose Meat Medium.
Adjust pH to 8.4
Boil for 20 minutes, restore to original volume with distilled water, filter through paper until clear.
Add 0.2% dextrose.

The chopped cooked meat is washed in strainer under running water to remove fine particles, allowed to drain, and excess water squeezed out.

The cooked meat medium is to be distributed into tubes, 8" x 1", filled $\frac{1}{4}$ full with cooked meat, and at least 35cc. of broth added.

Tubes are autoclaved at 15 lbs. pressure for 30 minutes. Final pH should be 7.4 to 7.6.

### B. Anaerobic cultures:

5cc. of the plasma from the pool is incubated into an 8" x 1" tube containing 40ccs. Brewer's dextrose Thioglycolate medium (A.M.A. 115:598, August 24, 1940). This medium may be procured from the Baltimore Biological Laboratories in Baltimore, Md. It contains:

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| Pork Infusion solids (from 37.5 gms. pork) | 1.0% |
| Peptone-Thio | 1.0% |
| Dextrose | 1.0% |
| NaCl | 0.5% |

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| Sodium Thioglycolate | 0.1% |
| Agar | 0.05% |
| Methylene Blue (1:500,000) | 0.0002% |

Dissolve 3.65 grams in 100cc. of distilled water. Heat until the solution boils and allow to boil about one minute. Autoclave 20 minutes at 120°C, 40cc. to each tube. Do not store the powder in the refrigerator because this decreases the duration of anaerobiosis.

Both tubes are to be inoculated at 37°C for two weeks. Examination of standard preparations of these tubes are to be made after 3, 7 and 14 days. If bacteria are seen on stained preparation, transfusions of 0.5cc. are to be made to fresh 0.2% dextrose cooked meat media. (tubes 6" x 5/8"), and 0.2cc. is to be transferred to each of two blood agar plates, one for aerobic incubation and the other for anaerobic incubation. The organism should then be identified.

If at the end of 72 hours all cultures from each pool are negative, then that pool may be dispensed into final containers. If the 7-day or 14-day examination shows evidence of contamination not found at 72 hours, this should be reported to Dr. Frank Meleney's laboratory at the Presbyterian Hospital. Otherwise it will be assumed that the pool was free from contamination.

If the 72-hour examination of the pool shows a contaminant in both tubes, the pool is immediately discarded. If there is a contaminant in only one of the two tubes, 20ccs. of this pool should be recultured, (10cc. aerobically and 10cc. anaerobically). If after one week these cultures are sterile, the pool may be bottled.

II. <u>DISTRIBUTION OF POOL INTO FINAL BAXTER PLASMA BOTTLES.</u>

Immediately following the withdrawal of the sample of plasma from the fresh pool for culturing, there should be added to each pool sufficient buffered 1-100 merthiolate to form a dilution of

1-10,000 merthiolate in the final plasma-saline mixture. The safest way to be sure of the right proportion is to add 40ccs. of a freshly prepared 1% solution to each pooling flask containing 2000ccs. of plasma. A simpler method is to add 10ccs. of a 4% solution of merthiolate to each 2000ccs. pooling flask.

It has been suggested that the merthiolate powder be added to sterile distilled water and buffered by means of chemically pure sodium borate in the ratio of 1 gm. merthiolate to 1.4 gm. sodium borate per 100cc. distilled water. Or, in case of the stronger solution, 4 gm. merthiolate to 5.6 gm. of sodium borate per 100cc. distilled water.

Such pools are then set aside in the refrigerator until the 72-hour culture reports are returned. If these reports are negative, then each pool may be dispensed into the final containers. Each Plasmavac contains 500 cc. physiological saline, to which is added 500 cc. of the pooled plasma. The last 35cc. of plasma in the pool is run into a special control bottle containing 35cc. of normal saline from the same batch of normal saline used in the plasmavacs. This special bottle is to be labelled with the date, the name of the hospital, the number of the pool, and the carton number in which the pool is packed. These control bottles will be sent with the carton to the warehouse where a messenger will pick them up each day to carry them to the central laboratory, which is situated in the surgical bacteriological laboratory of the Presbyterian Hospital. Here rechecks on the pools will be done at 3-day, one-week, and two-week intervals, before the cartons are finally released for shipment.

Methods are now being worked out for over-coming the

bacteriostatic action of the merthiolate. This is the problem of the central laboratory, and does not affect the routine in the individual hospital laboratories at this time. The ammonium sulphide procedure for neutralizing merthiolate is to be entirely discarded.

III. POSITIVE CULTURES IN CENTRAL LABORATORIES FOLLOWING NEGATIVE CULTURES IN HOSPITAL LABORATORIES.

Should a positive culture be obtained in the central laboratory on a plasma pool which has already been released by the hospital and sent to the store-room, the whole pool will be brought to the central laboratory and each flask in that pool will be separately cultured. The hospitals therefore should have readily available records of the serial numbers of the plasmavac bottles which were put up from any given pool.

The same procedure will be carried out if the one-week or two-week findings on the individual hospitals should be positive following a negative 72-hour culture. This information is to be transferred to the central laboratory at once so that such pools may be retrieved from the store-house and re-cultured.

IV. TOXICITY TEST

In order to add a factor of safety and to come within the spirit of the law, one mouse will be injected intra-peritoneally with 1cc. of plasma from each control bottle, and observed for 72 hours. It is not necessary for this toxicity test to be done by the individual hospitals.